Progress Report – FY2005

Core Name: Pathogen Source Tracking

Project Title: Adapting Luminex to Microbial Source Tracking

Reporting Period: 1 October 2005 through 30 September 2006

Principal Investigator(s): Kelly D. Goodwin, Atlantic Oceanographic and Meteorological Laboratory

Associate Investigator(s): Jack Fell, University of Miami Rosenstiel School of Marine and Atmospheric Science

Background and Rationale:

Source tracking is a critical need in water quality management. Coastal waters contaminated by fecal pollution have deleterious consequences to human and ecosystem health and to the economy. Water quality managers require both rapid water quality data and information about the sources of impairment. This data is needed to properly guide remediation strategies and to formulate policies founded in science. Current monitoring techniques whether for HABs or fecal contamination are too slow and labor intensive, which makes environmental study and management difficult. The methods presently used to monitor recreational water quality (e.g., *E. coli* or *Enterococcus* spp. CFU or MPN) do not yield information regarding human versus animal sources because the organisms enumerated in the methods are not host specific. In addition, indicator counts can show extreme variation which further motivates the need to locate the spatial source of contamination so that adequate remediation strategies can be devised.

The Luminex xMAP^{TM} system is a powerful tool with the potential to meet source tracking needs. It is essentially a flow cytometer equipped with two lasers, one that identifies a color-coded bead (there are 100 available) and the other that registers whether or not the capture probe has captured a target (e.g., DNA, RNA, protein, antigen). The system works in a 96-well plate format. In each well, detection of multiple targets can be achieved, and each well is read rapidly – in about 45 seconds. This project aims to adapt this technology, designed for clinical applications, to coastal water quality monitoring applications.

Objectives:

- Develop a high throughput DNA hybridization assay to detect sewage indicating bacteria and source tracking markers.
- In communication with other microbiology core members, optimize the upstream processes of nucleic acid extraction and amplification.
- Aid development of a database system that will allow tracking of environmental and molecular data in a coordinated fashion within and between various laboratories.

Accomplishments:

- The following manuscript has been completed has been submitted: "Luminex Detection of Fecal Indicators in River Samples, Marine Recreational Water, and Beach Sand." I.B. Baums, K.D. Goodwin, T. Kiesling, D. Wanless, and J.W. Fell.

- The Luminex assay as described in the manuscript above has been substantially redesigned in order to increase sensitivity and specificity. Changes include more specific priming (removal of the universal reverse primer), optimization of the multiplex PCR amplification to increase sensitivity (optimization of primer concentrations and annealing conditions, designing amplicons to be of more similar length), and switching of amplification targets to increase specificity of priming with environmental samples (e.g., 23S of *Enterococcus* spp. rather than 16S).

- The following source tracking markers were incorporated into Luminex analysis: the human-associated *esp* gene of enterococci, the human-specific HF8 marker of *Bacteroides* spp., and a dog-specific marker designed for the primers DF475F/Bac708R. Detection of HF8 has been successfully achieved in environmental samples. The requirement of a preculture step to achieve detection of the *esp* gene was confirmed. Adequate sensitivity was not achieved with the dog-specific marker, and that marker has been redesigned. Further testing is funding-dependent.

- DNA extraction efficiency experiments revealed poor recovery with standard DNA spin kits (0-2%). Extraction protocols have switched to bead beating to generate crude lysate. However, extraction efficiency remains unacceptably low (0-39%), and work continues in this area.

- Water processing protocols now incorporate a culture step on selective media in order to increase sensitivity of the assay to source tracking markers.

- The FileMaker Pro database has been completed. Initial code has been refined and simplified to make it more robust and more easily adaptable to HML needs. The database is now being beta-tested, particularly with regard to report generation.

- AOML and HML personnel have worked successfully as a team, despite physical distance, in order to maximize the work that can be accomplished under an unfavorable funding climate.

Publications/Presentations:

K.D. Goodwin Garcia, C., Bonilla, J.A., Bonilla, T.D., Wanless, D., Abdelzaher, A. LaGier, M.J., Solo-Gabriele, H., Concentration and Extraction: Efforts to Overcome Issues with Processing Coastal Water Samples for Downstream Molecular Analysis. American Society of Microbiology, Florida Branch Meeting, Cocoa Beach, FL, March 31-April 1, 2006.

K.D. Goodwin. Next Generation Sensors for the Integrated Ocean Observing System. Public Health Risks: Coastal Observations for Decision Making, St. Petersburg, FL, Jan. 23-25, 2006.

K.D. Goodwin. Adapting the Luminex System for Water Quality Analysis. Oceans & Human Health Initiative Principal Investigators Meeting, Charleston, SC, Jan. 18-20, 2006.

J. Gooch, K. Goodwin, J. Gregory, J. Jacobs., J. Lewis, W. Litaker, B. Robinson, J. Stewart. Concentration, Extraction and Detection: Efforts to Overcome Common Issues with Isolating Microbes from Environmental Samples. Oceans & Human Health Initiative Principal Investigators Meeting, Charleston, SC, Jan. 18-20, 2006.

K.D. Goodwin. Adaptation of the Luminex 100 System to Use in Recreational Waters, Sustainable Beaches Conference '05, St. Petersburg, FL, Oct. 31-Nov. 2, 2005.

M.E. Durbin, A.M. Zaher, N.F. Heybeck, H.M. Solo-Gabriele, S. Elmir, K.D. Goodwin, C. Sinigalliano. The Inter-Tidal Zone is the Source of Enterococci to a Subtropical Recreational Beach. ASM, Atlanta, GA, June 5-9 2005.

K.D. Goodwin. Development of Molecular Biological Tools for Monitoring Coastal Water Quality Monitoring, Sea Tech, Dania, FL, March 17, 2005.

Application/Technology Transfer relevant to OHH Strategic Goals

1.0 Scientific Research and Application

Water quality managers require both rapid water quality data and information about the sources of impairment. These data are needed to properly guide remediation strategies and to formulate policies founded in science. This project aims to provide an innovative approach to monitoring coastal water quality for microbial contamination. It harnesses the power of biotechnology to rapidly detect multiple source tracking markers and indicator organisms simultaneously. In contrast, current source tracking methods either use a library-based approach (which has a variety of drawbacks) or utilize a series of separate analyses to detect host-specific markers. Fecal indicator detection is performed in additional separate steps using slow methods (>1 day) and focused on a single organism. The approach being developed here aims to rapidly detect a matrix of source tracking markers and indicator organisms, giving environmental managers comprehensive information on which decisions can be based. Coastal resource managers, environmental researchers, and aquaculture professionals are potential end-users of this technology. In addition to providing a management tool, this technology can be used as a research tool to increase the understanding of the causal relationships between humans, ocean processes, marine ecosystem health, and human health outcomes.

2.0 Public Information and Outreach

Our goal is to interact with the HML Education and Outreach program on technology transfer issues including lessons learned, contacts made, and ideas of how the program can provide information to developers, end-users, and stakeholders regarding technology transfer. In addition, this project will interface with the University of Miami NSF/NIEH Center for Oceans and Human Health: Dr. Goodwin is a member of the Internal Advisory Committee for the University of Miami RSMAS OHH Center. This project is integrated with on-going projects at that center and utilizes the outreach vehicles (website, science symposiums) enlisted by the RSMAS OHH Center.

3.0 Capacity Building

This project helps build the high-tech, interdisciplinary workforce needed to improve NOAA's ability to understand and respond to Oceans and Human Health related issues. Project goals include transfer or research to operations. Technology transfer resources are lacking within NOAA. Coordinating our efforts with the Education and Outreach will strengthen NOAA's capacities to transfer technology, and disseminate information.

Project abstract:

Microbial contamination and toxic algal blooms impact coastal water quality. As the nation's coastal areas become more urbanized, poor water quality has increasingly negative economic, health, and environmental impacts. Proper assessment and understanding of the factors that effect coastal water quality requires the ability to rapidly and simultaneously detect multiple microbial contaminants and determine their sources with regard to location and type of contamination. Such data informs mitigation strategies and health risk assessments. Environmental managers need quick and accurate measures of water quality so that they can restrict human access to contaminated marine waters and products. This project aims to harness biotechnology advances in the realm of clinical science and apply them to coastal water quality applications. We are adapting a DNA hybridization assay technology, the Luminex® 100™ to achieve this goal. The system is essentially a flow cytometer equipped with two lasers, one that identifies a color-coded bead (to which a molecular probe is attached) and the other that registers whether or not the probe has captured a target (DNA amplified by PCR, in this case). The system incorporates a suspension array that assays multiple analytes rapidly in a single well of a microtiter plate. The system works in a 96-well plate format. In each well, detection of multiple targets can be achieved, and each well is read rapidly – in about 45 seconds. Therefore, this system has the ability to provide rapid, multiplexed, high-throughput detection of biological organisms. We designed and field tested a variety of primer/probe sets to identify the DNA signatures of various bacteria that indicate fecal contamination and expanded our efforts to include molecular markers that indicate whether contamination is from animal or human sources. The Luminex response to cultures indicated that the system was specific and sensitive. The Luminex response to environmental samples was consistent with the number of bacterial cells available to

extract and results were consistent with DNA sequencing. The current array utilizes probes to simultaneously detect the following: the genus *Enterococcus, Bacteroides distasonis, Escherichia coli/Shigella* spp., the human specific HF8 cluster of *Bacteroides*, and the human-specific *esp* gene of *Enterococcus* spp. Overall, the data suggest that this technology has the potential to simultaneously detect multiple targets for coastal water quality applications, particularly as progress is made to efficiently extract DNA from water and sediment matrices.

Unresolved Issues:

- Primers and probes have been designed to further the progress of this project. In addition, this project (and the Microbiology group at HML) has a wonderful opportunity to participate in a survey of coastal sewage outfalls under the Florida Area Coastal Environment (FACE) program at AOML. Samples from outfall boil sites have been collected. Unfortunately, this project is stalled due to the current budget freeze and lack of assurance of continued funding.

Budget Report:

Personnel: (salaries prepaid to Cooperative Institute)	Yr1	Yr2	Yr3	Total
Postdoctoral Associate	40,700	42,735	0	83435
Research Associate	35,127	22,500	0	57627
hourly (1) @ $8.00/hr	5,500	4,074	0	9573.5
FileMaker programmer	0	3,200	0	3200
Fringe:				
Salaries @ 31.3%	23,734	21,420	0	45154.01
wages @ 9%	495	366.615	0	861.615
Indirect:				
Salaries and fringe @ 26%	27,445	24,517	0	51961.29
Total salaries, fringe, indirect:	133,000	118,812	0	251812.4
Supplies and Materials:				
including field sampling costs and database software	22,000	20,000	0	42000
Equipment:				
Total:	**$155,00**	**$138,812**		**$293,812**